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Patients with locally advanced breast cancer, which represents less than 5% of annually diagnosed breast cancer cases, are a very heterogeneous group of patients. The optimal management of these patients remains controversial. Several randomized and nonrandomized clinical trials demonstrate a benefit in the use of chemotherapy combined with local treatment (surgery and/or radiation therapy) for these patients with prolongation of both disease free and overall survival. Given the ability of radiation therapy to control local disease and the failure of conventional chemotherapy to provide long lasting control of distant disease, combining paclitaxel with radiation therapy seems worthy of investigation. We have shown in tissue culture a supraadditive interaction between this drug and radiation. In our clinical trial, patients received six weekly 3-hour infusions of paclitaxel during preoperative radiation therapy, followed by modified radical mastectomy when possible. All patients received adjuvant CAF chemotherapy. To date, we have enrolled six patients; three patients at 25 mg/M^2, and three patients at 33 mg/M^2 paclitaxel. No unexpected adverse reactions were observed in the first six patients. Three of six patients obtained a clinical complete response prior to surgery, two patients had a partial response, and one patient progressed. All patients had a mastectomy with a pathologic response remarkable for residual microscopic disease in those patients who had clinical complete responses. Two patients are dead of disease, three patients are free of disease, and one is alive, but clinical status is unknown. Pharmacokinetic studies revealed levels of drug present in serum 4-72 hours after infusion in the radiation sensitizing range observed in tissue culture. Needle aspirations of tumors during chemoradiation were obtained. Although results seem encouraging, i.e., good clinical response, more patients need to be enrolled. Efforts are underway to increase accrual.

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Peter R. Oelig 10/29/96

PI - Signature Date

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TITLE: PHASE I TRIAL COMBINING PACLITAXEL AND RADIATION THERAPY FOR TREATMENT OF LOCALLY ADVANCED BREAST CANCER

INVESTIGATOR: Peter B. Schiff, M.D., Ph.D.

INTRODUCTION

HYPOTHESIS

Laboratory Data from the Department of Radiation Oncology at this institution indicates a potential theraputic advantage of combining paclitaxel and radiation for some human tumor cell lines. The objectives of this clinical trial are to define and quantitate clinical toxicities using established toxicity scales of concomitant paclitaxel and radiation for women with locally advanced breast cancer.

BACKGROUND & SIGNIFICANCE

Treatment of Locally Advanced Breast Cancer

Locally advanced breast carcinoma accounts for only about 5% of newly diagnosed breast cancer and represents a very heterogeneous group of patients. In the early 20th century, the radical mastectomy developed by Halsted was the mainstay of treatment for all women with breast cancer. However, it soon became evident that not all women benefited from surgery. The pioneering work by Haagensen and Stout (1) based on their review of 1135 breast carcinomas treated at Columbia-Presbyterian Medical Center from 1915-1942 yielded a group of women whose disease was far advanced for surgical cure. The Columbia classification for operability was therefore developed (2). Radical mastectomy performed on these women was associated with a 53% local failure rate and a 0% five year disease free survival. In addition, the five "grave signs" were associated with increased likelihood of local recurrence, although each one separately did not represent inoperability. From that time on research has addressed itself to alternate modes of treatment for locally advanced disease. Baclese (3) reviewed his experience at the Foundation Curie from 1936-1945 using radiation alone, and was the first to treat locally with tumoricidal doses and protracted fractionation yielding clinical healing in 22/23 patients at three years for tumors less than 6 cm. and 8/42 with tumors >6 cm.

Fletcher and Montague also reported their extensive experience using radiation alone for locally advanced breast cancer giving 16% and 28% local failure rates respectively using 7 cm as the cutoff tumor diameter (4). Toxicities have been well reported with radiation alone (5) causing further investigation of combined modality approaches. Zucali and others have reported excellent local control using surgery and radiation, however distant metastatic disease remains a formidable problem with both modalities reporting an incidence of distant metastatic disease of 68% (6).

Few randomized trials, and several non randomized trials (7) have looked at the addition of chemotherapy in the management of locally advanced breast cancer and report prolongation of both disease free and overall survival. M.D. Anderson Hospital recently updated their experience (8) of 174 patients and reported a 87.4% response rate (16.7% complete) and 96.5% patients disease free after induction and local therapy. Overall and disease free survivals at three years was 84% and 71% respectively for stage IIIA versus 44% and 33% for stage IIIB patients. Distant disease continued to account for 70% of the failures. The Institute Gustave-Roussy also reported similar findings (9) with overall survival at three years at 70%. Approximately 50% of their patients failed because of metastatic disease.

Given the ability for radiation therapy to control disease locally and the failure of conventional chemotherapy to provide long lasting control of distant disease, combining paclitaxel with radiotherapy provides an avenue worthy

of investigation. Holmes, et. al. (10) have recently demonstrated activity of the drug in refractory metastatic breast cancer in patients who have failed standard chemotherapy.

Paclitaxel

Paclitaxel is a natural product isolated from the bark of the pacific yew, *Taxus brevifolia* . Its structure a novel diterpene compound, and antitumor activity in rodents were reported in 1971 (11). The drug's unique mechanism of action has generated considerable interest, both for its use to probe the function of the cytoskeleton in basic science, and as a chemotherapeutic agent in oncology. Unlike agents currently available (colchicine, podophyllotoxin, vinblastine, etc.) that bind to tubulin, the subunit of microtubules, and inhibit microtubule formation, paclitaxel induces the formation of exceptionally stable microtubules (12).

Tissue culture studies have shown the ability of paclitaxel to block and/or prolong cells in the G2 or M phase of the cell cycle (13). These observations probably explain the observed anti-tumor activity of the drug.

Radiation and drugs are now combined simultaneously in the treatment of several malignancies in an attempt to achieve improved local control which could lend to better relapse-free and overall survival. It is particularly attractive to combine agents with radiosensitizing activity and systemic activity. Paclitaxel has recently been shown to be a cell cycle selective radiosensitizer (14, 15). This effect is dependent on paclitaxel concentration and on the fraction of cells in the G2 or M phase of the cell cycle. The sensitizer enhancement ratio for 10 nM paclitaxel at 10% survival is approximately 1.8 and, for 1 nM paclitaxel, it is about 1.2. These results suggest that a significant advantage may derive from appropriate time-concentration dependent interactions in combined modality protocols.

Paclitaxel has undergone several phase I and II trials at many institutions (16). Plasma Concentrations of 0.01 to 1.0 M paclitaxel at safe therapeutic doses are comparable to those required for the antiproliferative and microtubule stabilizing effects of the drug *in vitro* . Mitotic arrest has been observed in biopsy specimens from esophagus, stomach, small intestine, colon, liver, skin, bone marrow, and testes of patients treated within 11 days after receiving paclitaxel (17).

BODY

METHODS

All patients will receive paclitaxel, diluted to the appropriate volume, through a free-flowing intravenous line. Paclitaxel will be given as a 3 hour infusion weekly (days 1, 8, 15, 22, 29, and 36), just before, and during radiation therapy. Infusions will be monitored by an oncology nurse, with supervision from a medical oncologist, who will also obtain vital signs every 15 minutes for the first hour then hourly during the infusion.

The starting dose of paclitaxel for the first three patients will be 25 mg/m^2. If well tolerated, then the dose will be escalated by 30% if there is no toxicity and 20% if there is less than or equal to grade 2 toxicity. The first patient will be followed through the entire paclitaxel/radiation phase of the protocol before entering additional patients. We expect to enter 3 patients at each dose level until MTD. If 0/3 patients develop dose limiting toxicity (DLT), the next dose level will be entered. If 1/3 patients develop DLT, an additional 3 patients will be treated at that dose level. If > 1/6 develop DLT then the dose will not be escalated. In any other circumstance the prior dose level will be considered the MTD.

If a patient demonstrates grade 3 or 4 leukopenia (WBC < 1000/ l), then G-CSF, 5 g/kg/day s.c. will be instituted in that cycle and will be continued until the WBC is ≥ 3000/ l. The next administration of Paclitaxel will be delayed for 1 week if WBC is not ≥ 3000/ l within 2 days prior to the scheduled paclitaxel retreatment day. At least 24 hours should elapse between the last dose of G-CSF and the next dose of paclitaxel. Thereafter, the patient will continue

to receive G-CSF starting on the day after paclitaxel administration and continuing for 5 days. If the WBC is not ≥ 3000/ l on the 5th day of G-CSF administration, G-CSF will be continued until the WBC is ≥ 3000/ l. The next administration of paclitaxel will be delayed for 1 week if WBC is not ≥ 3000/ l within 2 days prior to the scheduled paclitaxel retreatment day. At least 24 hours should elapse between the last dose of G-CSF and the next dose of paclitaxel. If a patient demonstrates intolerance to paclitaxel defined as grade 3 or 4 leukopenia refractory to G-CSF or occurrence of febrile neutropenia or neutropenic sepsis, additional Paclitaxel will not be administered until WBC ≥ 3000.

Radiation Therapy

Prior to initiation of radiation, appropriate treatment planning will be performed. This will include designing an individual immobilization device for the patient, simulation, and treatment planning CT. Wires will be placed around the palpable breast tissue. Tangential fields will be utilized to encompass the breast and underlying chest wall. The maximum acceptable lung volume as displayed on the simulation portal film is not to exceed 3 cm measured posteriorly from the central axis of the chest wall interface. If at all possible, the regional draining lymphatics will be incorporated into the tangential beam. The minimal volume encompassed by this field will be as follows: MEDIAL; 3 cm contralateral to midline. LATERAL; 2 cm inferior to the palpable breast tissue. SUPERIOR; at least 1 cm superior to palpable tissue; or the minimal volume that will encompass tumor and two centimeter margin. A separate supraclavicular portal will be designed which will abut the superior aspect of the medial tangential field and extend from the clavicular head to 1/3 the length of the clavicle. Radiation therapy would start 24 hours (day 2) after the first infusion of paclitaxel. All additional paclitaxel doses (days 8, 15, 22, 29, 36) will precede that days radiation treatment by at least 2 hours. All patients will be treated once daily, 5 times a week (4 times week 1 and 6) utilizing a 6 MV photon beam. This volume of target tissue will be brought to a total dose of 5040 cGy using 180 cGy fractions. If a patient develops significant moist desquamation in the radiation portal the patient will not receive additional radiation therapy until healing is established.

Clinical response will be assessed two weeks after paclitaxel/radiation. Complete Remission (CR): Complete disappearance of all evidence of tumor. Partial Response (PR): Tumor decreased by at least 50% in the sum of the products of the perpendicular diameters of all measured lesions in the absence of progression of any lesion or the appearance of any new lesions. Stable Disease (SD): Change in measurable disease too small to meet the requirements for partial response or progression and the appearance of no new lesions. Progression of Disease (PD): Development of any new areas of malignant disease or increase (>25%) in any pretreatment area of measurable tumor.

Surgery

Surgical evaluation will be performed within 2 weeks of completion of the paclitaxel/radiation phase of the study for possible modified radical mastectomy. If the surgeon is not confident of obtaining negative margins with surgery then the patient will receive additional radiation therapy. Nonsurgical patients will receive a total dose that will not exceed 7500 cGy. The modified radical mastectomy specimen or tissue obtained by needle aspiration from a patient's tumor that received 7500 cGy will receive a complete pathologic analysis. Pathologic response will be determined.

CAF Chemotherapy

Six cycles of conventional CAF (Cytoxan 500 mg/m^2 iv day 1, Adriamycin 50 mg/m^2 iv day 1, 5-FU 500 mg/m^2 iv day 1, q 3-4 wk) chemotherapy will be given following (within 6 weeks) the completion of paclitaxel/radiation or surgery. Patients that received 7500 cGy and 6 cycles of CAF will have a needle aspiration of their tumor, if possible, for complete pathologic analysis. G-CSF prophylaxis can be given on days 2 to 6 and days 9 to 14 at 5 g/kg/day.

During CAF chemotherapy patients will have weekly CBC's, a SMAC every 3 weeks and a CXR every 2 months.

Laboratory Studies

Quantitation of multiple drug resistance (mdr) gene in the peripheral blood of patients receiving paclitaxel by mdr polymerase chain reaction (PCR) and by FACS sorting using an antibody that is specific for mdr (Cetus 17F9) before treatment, during treatment, and after treatment.

Pharmacokinetics of paclitaxel using this dose schedule. Techniques will include HPLC (18, 19) and an ELISA or RIA method (20). Serum paclitaxel levels will be obtained at 30, 4, 8, 24, 72, and 96 hours post infusion weeks 1, 3, and 6.

A computerized image analyzer will be used to study estrogen and progesterone receptor expression, mdr, and tumor cell cycle parameters before, during, and possibly after treatment. Surgical biopsies and needle aspiration will be performed in the appropriate locations by the Surgeon(s) and/or by the Pathologist(s). Surgical biopsies will be immediately placed on ice and delivered to the Surgical Pathology Department by a dedicated messenger. The assigned Pathology attending/resident will macroscopically evaluate, ink and cut the specimen. Representative portions of fresh, unfixed tissue will be cut into tissue blocks and placed on top of cork discs covered with OCT-embedding compound and then snap frozen in a mixture of isopentane and dry ice and stored at - 80 C until needed. In addition, cryopreserved tissue will be used for frozen tissue section in order to document the presence of neoplastic cells in the pathological specimen and to perform additional immunohistochemical studies. A representative portion of each biopsy specimen will be fixed in formalin and embedded in paraffin for routine histopathology and for paraffin tissue section immunohistochemical studies.

Needle aspirate material will be used to perform smears on sialine pre-coated slides. The slides will be briefly air-dried and then appropriately fixed in buffered formalin, Zamboni and/or acetone-chloroform fixatives. At least two sections will stained in H&E to perform a cytological diagnosis. The syringe barrel will then be washed with media (RPMI-1640) containing 10% of fetal calf serum. The additional material will be collected after centrifugation and cytospin preparations will be performed and fixed as described above.

Estrogen and progesterone receptor expression will be evaluated using commercially available specific monoclonal antibodies (mABs) by an immunohistochemical technique. A similar technique will be used to measure p53. The percentage of positive cells and intensity of reactivity will quantitated using a computerized image analyzer (CAS 200) equipped with appropriate soft-ware programs. P-glycoprotein expression will also be evaluated using mABs (MRK16 and UC219) by an alkaline anti-alkaline (APAAP) immunohistochemical staining. In order to quantitate the p-glycoprotein expression, control slides prepared from glioblastoma cell lines carrying mdr-1 gene amplification(s) will be stained and used as positive control slides and p-glycoprotein expression will be quantitated and expressed as pg of mdr-1 protein product per cell.

The percentage of cycling cells will be evaluated using a mAB (Ki-67) which specifically recognize a cellular product expressed only in proliferating cells (G1b, S, G2 and M phase) by immunohistochemical assay. The percentage of positive cells will be quantitated by a computerized image analyzer equipped with the appropriated soft-ware program (Proliferation Index) and will be expressed as percentage of positive cells and/or percentage of positive nuclear area. The values obtained will also be compared with the data generated by Diploid analysis (see below).

Quantitative investigation with a computerized image analyzer will be used to evaluate the DNA content of a population of non-neoplastic and neoplastic cells. Calibration of the computer will be performed by measuring predeposited control cells with a known mass from which the mode of summed optical density will be obtained. The computer calculated a K value from which this data by linear equation: cell mass = K(•OD). S and G2-M fractions will be also calculated in normal as well as in neoplastic cells.

Tumor cell ultrastructure studies from needle aspiration samples or surgical specimens using electron microscopy to document expected microtubule cytoskeletal changes. Tissue preparation as described above plus fixation in 25% glutaraldehyde.

BIOSTATISTICAL DESIGN

Mr. Donald McMahon from the GCRC is the statiistician for the study. The study will be complete when the maximum tolerated dose of placlitaxel is obtained and will define and quantitate clinical toxicities using established toxicity scales. Pathological response to paclitaxel plus radiation will be reported.

Duration of Response: The duration of response will be defined as from the first date at which criteria were met to qualify patient as CR or PR until the first notation of clinical progression. Time to Disease Progression (Time to Treatment Failure): The time to disease progression will be the time from the first date of therapy until the first notation of clinical progression or relapse. A patterns of failure analysis will be performed to identify any possible changes compared to known patterns with conventional treatment.

HUMAN SUBJECTS INFORMATION

This protocol uses human subjects exclusively. The protocol will enroll approximately 25 women of all adult ages and from any ethnic background. Exclusionary and inclusionary criteria are stated clearly in our application which is approved by our IRB. We use no special classes of subjects. Research material will included blood, urine, and breast tissue. They will be obtained both for research purposes as well as for diagnosis and management. The consent procedures followed are entirely in conformity will the University guideline. There are no potential risks to confidentiality because of the nature by which the data are secured and used. The risks to the subjects are certainly reasonable in relation to the anticipated benefits and in relation to the importance of the knowledge that may reasonably be expected to result.

RESULTS

Six patients have been entered to date. All had AJC stage IIIb locally advanced breast cancer. The first three patients received 25 mg/M^2 paclitaxel 3-hour infusions every week during radiation therapy. The next three patients received 33 mg/M^2 3-hour infusions of paclitaxel. Mean follow-up time is 23.8 months with a range of 17 months. No significant toxicity ($\geq$ grade 3) has been observed in any of the six patients entered. Radiation reactions to skin and breast were no different than those encounter in patients receiving conventional breast irradiation. One patient (patient #1) had a transient (lasting <3 hours) patch of skin erythema in the affected breast during paclitaxel infusions, most likely allergic.

Kaplan-Meir cumulative survival for this group of patients is 53% at 34.5 months (2.9 years) [Appendix A]. Of the first six patients, three had a clinical complete response prior to modified radical mastectomy, with microscopic residual disease in the surgical specimen. Two patients had partial responses, while one patient developed supraclavicular adenopathy during paclitaxel/radiation treatment. Two patients are dead of disease at 17 and 26 months, four patients are alive between 17-34 months (3/4 patients have no evidence of disease, one patient is alive with unknown disease status). Of the first three patients, two had clinical CR s and one patient progressed during treatment and is dead of disease at 26 months. Among the second group of patients, one had CR and two had PR s [Appendix B].

Pharmacokinetic studies were performed on all six patients, using high pressure liquid chromotography (HPLC) and radioimmune assay (RIA). RIA was superior to HPLC in detecting paclitaxel serum levels in the nM range. For example, in patient #6 [Appendix C1F, C2F] HPLC could detect 5.7 nM paclitaxel while RIA could detect 0.1 nM paclitaxel. In addition, HPLC could not detect any drug in serum of patients after 24 hours, while the RIA could detect serum levels 96 hours post-placlitaxel infusion. The observed pharmacokinetics were similar to those previously reported (20). Using RIA, radiosensitizing levels of paclitaxel could be detected 72 hours post-infusion in some patients [Appendix C1A - C2F].

A possible cell cycle specific radiation sensitizer effect was shown when needle aspirations were obtained from tumors during chemoradiation and assayed for a variety of indices. For example, in some patients the percent of tumor cells in the G2-M phase of the cell cycle increased concomitantly with a decrease in the fraction of tumor cells in S-phase during the course of treatment consistent with the known mechanism of action of paclitaxel [Appendix D].

CONCLUSIONS

A phase I trial combining paclitaxel and radiation therapy for treatment of locally advanced breast cancer has been initiated and continues to accrue patients. No patients have experienced toxicity beyond grade II using the common toxicity criteria. An interim analysis of the first six patients reveals Kaplan-Meir cumulative survival of 53% at just under three years. This observed survival is similar to that reported in the literature for multimodality treatment of women with locally advanced breast cancer (8, 21). Two of the six patients are dead of disease at 17 and 26 months, while four patients are alive between 17 and 34 months. Three of the six patients had clinical complete responses with microscopic residual disease in the surgical specimen, while two had partial responses, with one patient having progression of disease during treatment. Pharmacokinetic studies using HPLC and RIA reveal radiosensitizing levels of paclitaxel in the serum of patients 72 hours post-infusion. Cell cycle tumor cell cycle changes were observed in patients that were consistent with the known mechanism of action of paclitaxel that were consistent with a possible cell cycle specific radiation sensitizer effect.

Although these early results with a few patients are encouraging, additional patients need to be entered.

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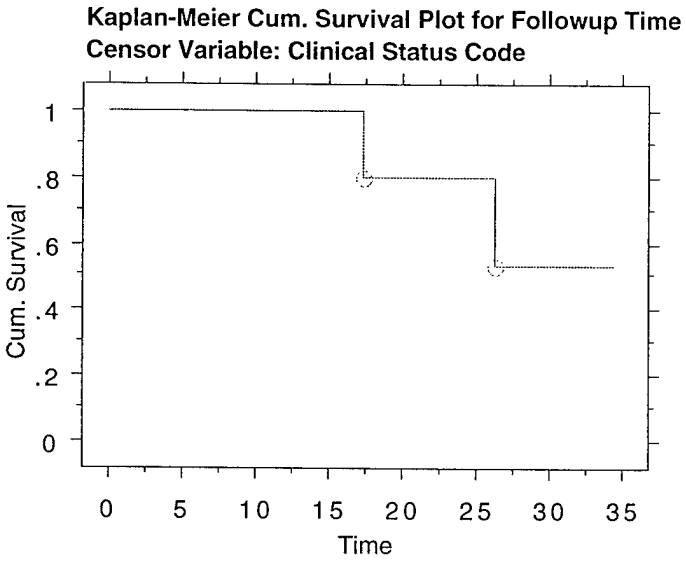
Appendix A


Kaplan-Meier Cum. Survival Plot for Followup Time
Censor Variable: Clinical Status Code
Cum. Survival
1
.8
.6
.4
.2
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5
10
15
20
25
30
35
Time

Appendix B

CPMC - RO 92-01 / NCI T92-0059

Patient Number	AJC Stage	Paclitaxel Dose	Clin/Path Res	F/U Mos	Vital Status
1	IIIB	25mg/M^2	CR/PR	17	DOD
2	IIIB	25mg/M^2	CR/PR	32	A/NED
3	IIIB	25mg/M^2	POD	26	DOD
4	IIIB	33 mg/M^2	PR	17	A/NED
5	IIIB	33 mg/M^2	PR	34	A/NED
6	IIIB	33 mg/M^2	CR/PR	17	A*

CR - complete response
PR - partial response
POD - progression of disease

DOD - dead of disease
NED - no evidence of disease
A - alive

* Clinical Status unknown

Appendix C1A

Patient 1

Assays by HPLC for Paclitaxel in Patient Serum (Breast Cancer) Treated with Paclitaxel and Radiotherapy (nM)*

Protocol: Continuous IV Infusion of 25 mg/m2 of Paclitaxel for Three Hours

Samples (Hours after Paclitaxel infusion)**	**Week 1**	**Week 3**	**Week 6**
4 hours	21.2	24.2	24.1
8 hours	ND	ND	ND
24 hours	ND	ND	ND
72 hours	ND	ND	ND
96 hours	ND	ND	ND

* Each value is the average of an assay in duplicate.
** Prior to treatment with Paclitaxel, level in serum was in accord with absence of Paclitaxel (i.e. negative control)
ND None detected

Appendix C1B

Patient 2

Assays by HPLC for Paclitaxel in Patient Serum (Breast Cancer) Treated with Paclitaxel and Radiotherapy (nM)*

Protocol: Continuous IV Infusion of 25 mg/m2 of Paclitaxel for Three Hours

Samples (Hours after Paclitaxel infusion)**	**Week 1**	**Week 3**	**Week 6**
4 hours	ND	ND	24.1
8 hours	ND	ND	12.8
24 hours	ND	ND	ND
72 hours	ND	ND	ND
96 hours	ND	ND	ND

* Each value is the average of an assay in duplicate.
** Prior to treatment with Paclitaxel, level in serum was in accord with absence of Paclitaxel (i.e. negative control)
ND None detected

Appendix C1C

Patient 3

Assays by HPLC for Paclitaxel in Patient Serum (Breast Cancer) Treated with Paclitaxel and Radiotherapy (nM)*

Protocol: Continuous IV Infusion of 25 mg/m2 of Paclitaxel for Three Hours

Samples (Hours after Paclitaxel infusion)**	**Week 1**	**Week 3**	**Week 6**
4 hours	37.3	21.2	21.9
8 hours	30.5	16.8	ND
24 hours	22.2	ND	ND
72 hours	ND	ND	ND
96 hours	ND	ND	ND

* Each value is the average of an assay in duplicate.
** Prior to treatment with Paclitaxel, level in serum was in accord with absence of Paclitaxel (i.e. negative control)
ND None detected

Appendix C1D

Patient 4

Assays by HPLC for Paclitaxel in Patient Serum (Breast Cancer) Treated with Paclitaxel and Radiotherapy (nM)*

Protocol: Continuous IV Infusion of 33 mg/m2 of Paclitaxel for Three Hours

Samples (Hours after Paclitaxel infusion)**	**Week 1**	**Week 3**	**Week 6**
4 hours	ND	28.5	27
8 hours	ND	ND	18
24 hours	ND	ND	ND
72 hours	ND	ND	ND
96 hours	ND	ND	ND

* Each value is the average of an assay in duplicate.
** Prior to treatment with Paclitaxel, level in serum was in accord with absence of Paclitaxel (i.e. negative control)
ND None detected

Appendix C1E

Patient 5

Assays by HPLC for Paclitaxel in Patient Serum (Breast Cancer) Treated with Paclitaxel and Radiotherapy (nM)*

Protocol: Continuous IV Infusion of 33 mg/m2 of Paclitaxel for Three Hours

Samples (Hours after Paclitaxel infusion)**	**Week 1**	**Week 3**	**Week 6**
4 hours	33.5	27.4	11.1
8 hours	18.5	22.0	ND
24 hours	10.9	10.6	ND
72 hours	ND	ND	ND
96 hours	ND	ND	ND

* Each value is the average of an assay in duplicate.
** Prior to treatment with Paclitaxel, level in serum was in accord with absence of Paclitaxel (i.e. negative control)
ND None detected

Appendix C1F

Patient 6

Assays by HPLC for Paclitaxel in Patient Serum (Breast Cancer) Treated with Paclitaxel and Radiotherapy (nM)*

Protocol: Continuous IV Infusion of 33 mg/m2 of Paclitaxel for Three Hours

Samples (Hours after Paclitaxel infusion)**	**Week 1**	**Week 3**	**Week 6**
4 hours	11.5	13.8	17.6
8 hours	8.8	11.5	13.2
24 hours	5.7	8.4	ND
72 hours	ND	ND	ND
96 hours	ND	ND	ND

* Each value is the average of an assay in duplicate.
** Prior to treatment with Paclitaxel, level in serum was in accord with absence of Paclitaxel (i.e. negative control)
ND None detected

Appendix C2A

Patient 1

Assays by RIA for Paclitaxel in Patient Serum (Breast Cancer) Treated with Paclitaxel and Radiotherapy (nM)*

Protocol: Continuous IV Infusion of 25 mg/m2 of Paclitaxel for Three Hours

Samples (Hours after Paclitaxel infusion)**	**Week 1**	**Week 3**	**Week 6**
4 hours	109	126	88.0
8 hours	51.0	24.8	46.7
24 hours	30.6	16.0	9.00
72 hours	1.49	1.94	1.21
96 hours	0.44	0.48	0.71

* Each value is the average of an assay in duplicate.
** Prior to treatment with Paclitaxel, level in serum was in accord with absence of Paclitaxel (i.e. negative control)

Appendix C2B

Patient 2

Assays by RIA for Paclitaxel in Patient Serum (Breast Cancer) Treated with Paclitaxel and Radiotherapy (nM)*

Protocol: Continuous IV Infusion of 25 mg/m2 of Paclitaxel for Three Hours

Samples (Hours after Paclitaxel infusion)**	**Week 1**	**Week 3**	**Week 6**
4 hours	25.4	37.2	26.5
8 hours	17.5	14.6	7.09
24 hours	7.42	4.87	4.02
72 hours	0.67	No sample	1.59
96 hours	0.40	0.63	No sample

* Each value is the average of an assay in duplicate.
** Prior to treatment with Paclitaxel, level in serum was in accord with absence of Paclitaxel (i.e. negative control)

Appendix C2C

Patient 3

Assays by RIA for Paclitaxel in Patient Serum (Breast Cancer) Treated with Paclitaxel and Radiotherapy (nM)*

Protocol: Continuous IV Infusion of 25 mg/m2 of Paclitaxel for Three Hours

Samples (Hours after Paclitaxel infusion)**	**Week 1**	**Week 3**	**Week 6**
4 hours	19.5	22.5	40.7
8 hours	14.3	14.2	35.2
24 hours	5.50	5.63	13.3
72 hours	2.60	2.59	3.28
96 hours	0.67	1.14	No sample

* Each value is the average of an assay in duplicate.
** Prior to treatment with Paclitaxel, level in serum was in accord with absence of Paclitaxel (i.e. negative control)

Appendix C2D

Patient 4

Assays by RIA for Paclitaxel in Patient Serum (Breast Cancer) Treated with Paclitaxel and Radiotherapy (nM)*

Protocol: Continuous IV Infusion of 33 mg/m2 of Paclitaxel for Three Hours

Samples (Hours after Paclitaxel infusion)**	**Week 1**	**Week 3**	**Week 6**
4 hours	19.8	44.9	42.0
8 hours	11.6	No sample	31.5
24 hours	6.15	9.11	11.8
72 hours	0.81	2.98	3.14
96 hours	0.22	2.21	No sample

* Each value is the average of an assay in duplicate.
** Prior to treatment with Paclitaxel, level in serum was in accord with absence of Paclitaxel (i.e. negative control)

Appendix C2E

Patient 5

Assays by RIA for Paclitaxel in Patient Serum (Breast Cancer) Treated with Paclitaxel and Radiotherapy (nM)*

Protocol: Continuous IV Infusion of 33 mg/m2 of Paclitaxel for Three Hours

Samples (Hours after Paclitaxel infusion)**	**Week 1**	**Week 3**	**Week 6**
4 hours	9.0	22.7	9.6
8 hours	7.0	6.5	5.9
24 hours	2.8	< 1	3.1
72 hours	< 1	< 1	< 1
96 hours	< 1	< 1	No sample

* Each value is the average of an assay in duplicate.
** Prior to treatment with Paclitaxel, level in serum was in accord with absence of Paclitaxel (i.e. negative control)

Appendix C2F

Patient 6

Assays by RIA for Paclitaxel in Patient Serum (Breast Cancer) Treated with Paclitaxel and Radiotherapy (nM)*

Protocol: Continuous IV Infusion of 33 mg/m2 of Paclitaxel for Three Hours

Samples (Hours after Paclitaxel infusion)**	**Week 1**	**Week 3**	**Week 6**
4 hours	16.6	20.8	14.3
8 hours	10.7	8.8	10.9
24 hours	6.3	5.4	2.8
72 hours	1.4	0.3	0
96 hours	0.1	0	No sample

* Each value is the average of an assay in duplicate.
** Prior to treatment with Paclitaxel, level in serum was in accord with absence of Paclitaxel (i.e. negative control)

Appendix D

Results from Needle Aspirations

Patient and Date(s) of Aspirations	DNA	S	G2-M	PI	ER	PR	HER-2/NEU
Patient 1							
7/13/93	1.0	17.5%	31.3%	18.2%	2.6%	2.1%	0.22
4/30/93	1.1	3%	6%	1.5%	2.9%	1.7%	0.22
Patient 2							
1/21/94	1.95	4%	-	4.3%	58%	56%	0.26
Patient 3							
2/8/94	1.7	10.5%	-	20%	6.2%	4%	1.0
11/29/93	2.07	7%	-	8%	7.4%	1.3%	.85
Patient 4							
4/19/94	1.4	3%	14.5%	2%	42%	38%	.14
Patient 5							
3/31/95	1.7	22.4%	8%	14%	52%	81%	.0002
12/27/94	2.05	9%	4%	13%	49%	45%	.41
Patient 6							
4/25/95	1.09	21.6%	17%	21.6%	6%	4%	.18